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(71) Applicant: THE VAN KAMPEN GROUP, INC. [US/US]; 881 East 5550 South, Ogden, UT 84405 (US).

(72) Inventors: VAN KAMPEN, Kent; 1008 Lake Winds Drive, Hoover, AL 35244 (US). EDWARDS, Bobby, G.; 19448 FM 2115, Salado, TX 76571 (US). FRANKLIN, Lanny, U.; 5170 Chemin de Vie, Atlanta, GA 30342 (US). HYMAS, Theo; 13855 Via Encantado, Valley Center, CA 92082 (US).

(74) Agents: LAURENZO, Brian, J. et al.; Dorsey & Whitney LLP, Suite 3900, 801 Grand, Des Moines, IA 50309 (US).

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(54) Title: THE USE OF SPECIFIC TERPENES TO KILL, IMPEDE, RENDER INACTIVE, OR INHIBIT THE GROWTH OF PLANTS, BACTERIA, FUNGI, YEASTS, VIRUSES, AND INSECTS, PARASITES

(57) Abstract

The use of β–ionone (a naturally occurring compound, chemically classified as a terpene) alone or in combination with other terpenes, biologicaly actives substances, and surfactants to inhibit growth, proliferation, impede such, render inactive or kill certain classes of plants, bacteria, fungi, yeast, viruses, parasites and insects. Wherein varying combinations of β–ionones in the above mentioned combinations have been demonstrated to kill braod leaf plants and grasses, impede fluorescence, budding, rooting of certain plants; whereas such combinations kill, impede metamorphosis, stun, incapacitate selected insects and parasites; wherein β–ionone kills anaerobic bacteria that are pathogens of live stock and man; the combinations kill vanthomillus, pseudomonas and other pathogenic bacteria affecting plants; wherei n the combination kills fungi, specific candidia, microspora and others infecting man and animals, the invention serves multiple functions as a means to control or kill certain pests or pathogens. β–ionone is non–toxic to humans and when exposed to the natural environment undergoes breakdown (Degradation) into carbon dioxide and water providing an environmentally compatible, safe and efficacious control of pests and pathogens.

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The Use of Specific Terpenes to Kill, Impede, Render Inactive, or Inhibit the Growth of Plants, Bacteria, Fungi, Yeasts, Viruses, Parasites and Insects

Background of the Invention

Field of the Invention

The present invention relates to the use of specific terpenes to kill, inhibit or regulate the growth of certain plants, bacteria, fungi, yeasts, viruses or parasites, and to kill, impede, repel or render inactive certain insects and snakes. The terpenes may be used in combination with any or all of other terpenes, surfactants, water and alcohol.

Background Art

Historically, terpenes have been isolated from green plants as an unsaturated hydrocarbon occurring in the oils and oleoresins. Nevertheless, new compounds structurally related to terpenes continue to be isolated from other sources as well. Of significant importance to the present invention is that a pathway in plants, animals, and insects known as the mevalonate pathway is interrupted by terpenes, specifically β-ionone. β-ionone is known to accelerate plant growth, inhibit the sporangial germination of different strains of bacteria, inhibit aflatoxin production, inhibit some species of fungi and causes carotenogenesis in others, be a powerful chemoattractant for insects, lower the cholesterol levels in animals, and have anti-tumor action. It is also known to prevent infections or killing of malaria or giardia and other intestinal pathogens or parasites in man.

β-ionone belongs to a family of mevalonate derived compounds, some members of which have been shown to have growth regulatory properties in higher plants and fungi.

Exogenous β-ionone has been reported to alter patterns of terpenoid metabolism in *Blakeslea trispora* (Feofilova, E.F. and Arbuzov, V.A. (1975), *Microbiology,* 44:351-354) by apparently enhancing translation of mRNA for enzymes that are functional early in the mevalonic acid pathway (Salt, S.D., Tuzun, S. and Kue, J. (1986) *Physiol. Molec.Plant Path.* 28:287-297). β-ionone, an end-ring analog of β-carotene, and its oxygenated analogs are widely distributed in plant products in free forms. However, the origin of β-ionone is not clear. Condensation of citral with acetone yields pseudo-ionone; treatment of pseudoionone with dilute sulfuric acid then yields a 50/50 mixture of α- and β-ionone. According to Weeks (Weeks, W. W. (1986), *ACS Symp. Ser. 317:157-166. Am. Chem. Soc.*, Washington, DC.), β-carotene, the most widely known tetraterpene in plants, is the direct precursor of β-ionone. The possibility of the bioconversion of β-carotene to β-ionone, the in situ synthesis of β-ionone or the hydrolysis of β-ionone conjugates is suggested by the finding that the concentration of free β-ionone in green tissues of tobacco plants increases 600-fold in response to immunization with a blue mold pathogen, *Peronospora tabacina* or tobacco mosaic virus (Salt, S.D., Tuzun, S. and Kue, J. (1986), *Physiol. Molec. Plant Path.* 28:287-297). β-ionone accelerates plant growth (Salt, S.D., Tuzun, S. and Kue, J. (1986), *Physiol. Molec. Plant Path.* 28:287-297), inhibits the sporangial germination of *Peronospora tabacina* (Salt, S.D., Tuzun, S. and Kue, J. (1986), Physiol. Molec. Plant Path. 28:287-297), *Aspergillus flavus* (Wilson, D. M., Guelkner, R. C., McKinney, J. K., Liersay, R.H., Evans, B.B. (1981), *JAOCS*, 58:959A-961A.21) and *Aspergillus parasiticus* (Wei, C. I., Tan, H., Fernando, S. Y. and Ko, N-J. (1986), *J. Food Protect.* 49:515-518 and inhibits aflatoxin production (Wei et al. (1986); Wilson, D. M. Jr. and Gueldner, R. C.

(1984), *Control of Mycotoxin Production by Chemically Inhibiting Fungal Growth*, U.S. Patent No. 4,474,816). β-ionone is stimulatory of carotenogenesis in some species of fungi and inhibitory in others (Wilson et al. (1984). Apart from these actions, β-ionone is a powerful chemoattractant for some species of insects (Donaldson, J. M. I., McGovern, T.P. and Ladd, T.L. Jr. (1990), *J. Econ. Entomol.*, 83:1298-1305; Lampman, R. L. and Metcalf, R. L. (1988), *Environ. Entomol.* 17:644-648). Secondary products of plant mevalonate metabolism suppress the synthesis of mevalonate, the rate-limiting substrate for the synthesis of isoprenoid intermediates essential for cell proliferation.

The use of terpene derivatives as antifungal agents is well known and is taught in prior patent literature. For example, U.S. Patent No. 4,963,583 and U.S. Patent No. 5,001,155 both teach the use of β-ionone derivatives as anti-fungal agents. U.S. Patent No. 4,474,816 teaches a method for inhibiting aflatoxin produced by strains of *Aspergillus parasiticus* fungi by the application of β-ionone. U.S. Patent No. 4,814,163 discloses an anti-tartar mouth deodorant comprising a zinc compound, an ionone ketone terpene derivative, and a mint flavor as the essential active ingredients. Yet another anti-tartar mouth deodorant comprising a zinc compound, an ionone ketone terpene derivative and a flavor as the essential active ingredients is disclosed in U.S. Patent No. 4,814,164. U.S. Patent No. 5,610,196 teaches the use of some terpenes and terpenones as topical antimicrobial agents.

Thus, while the prior art teaches the use of terpenes and/or terpene derivatives as active ingredients for controlling some bacteria and fungi, there is no teaching in the prior art of such terpene composition useful to kill specific bacteria and fungi pathogenic to plants, as a herbicide for the killing of broad leaf plants and grasses that commonly grow

as weeds, or for impeding the growth or fluorescence of such weeds. The prior art does not teach the use of such terpene compositions as an insecticide to kill or repel specific insects such as sicilian wasps, cockroaches, palmetto bugs, bees, spiders, certain ants, and flies.

Summary of the Invention

The present invention provides compositions comprising about 1-99.9 %, by volume, a reagent comprising an ionone composition of at least one ionone, another terpene, surfactants, and alcohol, with water, and methods for using such compositions.

The ionone composition in the reagent comprises ionones such as β-ionone, α-ionone, and pseudo-ionone, alone or in combination with each other. The terpene may be DL-limonene, dipentene, citral, terpineol or pinene. The surfactant is selected from silicons, polysorbate or tween-based surfactants. Alcohols such as ethyl alcohol and isopropyl alcohol may be used in the reagents.

The ionone based compositions of the present invention may be used to kill or inhibit the growth of bacteria, fungi, viruses, yeasts, and parasites; to kill or repel insects and snakes; to selectively kill weeds found in fields, turf, flower beds, and soil; and to control the fluorescence of flowering plants and regulate the growth rate of plants. The reagents of the present invention may be used as fungicides, herbicides, insecticides, fly and snake repellants, and plant growth regulators.

Description of the Invention

The present invention relates to the use of a terpene such as β-ionone, alone or in combination with other terpenes, biologically active substances, surfactants, alcohol and water to inhibit the growth, prevent the proliferation, and/or kill certain classes of plants,

bacteria, fungi, yeast, viruses, parasites; to impede, render inactive or kill certain classes of bacteria, fungi, yeast, viruses, parasites and insects; to repel certain varieties of insects and snakes; and to regulate the growth of certain varieties of plants. Varying the levels of β-ionone in the above mentioned combinations have been shown to kill broad leaf plants and grasses and impede the fluorescence, budding, or rooting of certain plants. Additionally, these combinations can kill, stun, and incapacitate selected insects and parasites. These insects include sicilian wasps, bees, spiders, and certain ants. It has also been demonstrated that β-ionone, alone or in combination with other terpenes or other biologically active substances, surfactants, alcohols, water, *etc.*, kills anaerobic bacteria that are pathogens of livestock and man. Specifically, the combinations kill *vanthomillus*, *pseudomonas* and other pathogenic bacteria affecting plants, and fungi, specifically *candidia*, *microspora* and others infecting man and animals. The invention inhibits the growth of fungi, bacteria, and yeast in fruit and fruit trees. The invention has been used to prevent and control the occurrence of walnut blight in walnut trees, and to control brown rot, blossom blight or fruit rot in certain varieties of fruits and fruit trees. The invention inhibits the growth of *Erwinia sp.* and *Xanthomonas sp.*, bacterial infections found in walnut trees and fruit trees. The invention inhibits the growth of bacteria in cuttings taken from fruit bearing plants, such as strawberry runners. The invention displays strong anti-viral activity against viruses, such as tobacco mosaic virus. The invention serves multiple functions as a means to control or kill certain pests or pathogens.

Through laboratory tests, it was discovered that both β-ionone and pseudo-ionone begin to have an effect against several types of bacteria at approximately 20 ppm.

However, at concentrations of greater than 100 ppm nearly 100% of both bacteria and fungi are killed in controlled experiments. This is significant because it is far less than the toxic level (LD_{50} in mice is greater than 5 g/kg) and far below a level that would normally be expected to cause eradication of the bacteria and fungi. Experiments were conducted to maximize efficacy and to create a practical and usable format for the ionones. Alpha-ionone was found to have almost no efficacy.

Because of the safety of β-ionone and pseudo-ionone, and because of the necessity of delivering the active ingredients in a water solution, it was determined that a non-ionic surfactant having an HLB range of 10 to 18 was necessary. Depending on the specific use, the preferred surfactants are polysorbate, silicons, and tween based surfactants.

The addition of another terpene to β-ionone was found to increase the efficacy when used in combination with either the beta-ionone or the pseudo-ionone. The terpenes tested included DL-limonene, dipentene, citral, terpineol, or pinene. All the aforementioned terpenes proved to be effective. However, DL-limonene was chosen because of its "generally regarded as safe" ("GRAS") status.

Since the final mixture of β-ionone or pseudo-ionone with DL-limonene and polysorbate forms a cloudy mixture, isopropyl alcohol was added to give a clear appearance. The isopropyl alcohol appears to have no bearing on the activity of the two principal active ingredients.

Depending on the prescribed use, different preferred compositions and/or formulations of β-ionone may be used. Formulations generally used for insecticidal and herbicidal purposes include 5-10% β-ionone with 70-95% d-limonene. These

compositions may also contain a surfactant and alcohol. The compositions may be further diluted in water depending on the strength desired.

The combinations used to inhibit the fluorescence of certain flowering plants include a β-ionone mixture of 85% β-ionone and 15% Tween-80 which is then dissolved at levels of 0.25% and 0.5% in water. For the inhibition of growth of *Erwinia sp.* and *Xanthomonas sp.*, bacterial infections found in walnut trees and fruit trees, β-ionone was serially diluted with reagent grade ethanol to create test solutions of 0, 33, 100, and 333 ppm. 694-B, a β-ionone/terpene formulation consisting of about 60% β-ionone, 15% Tween-80 (or a suitable surfactant) and about 25% of an additional terpene like α-terpenol is used to reduce the incidence and severity of walnut blight on walnut trees and the prevention of brown rot in stone fruit trees bearing such fruits as peaches and nectarines. Combinations of terpenes (*e.g.*, β-ionone and DL-limonene, optionally with a surfactant and/or alcohol) may also be used to treat fields prior to planting crops to kill or reduce the incidence of fungi, and kill weeds. These combinations may also be used to kill weeds and fungi in turf areas like lawns, *etc.*

β-ionone is non-toxic to humans and degrades into carbon dioxide and water when exposed to the natural environment, thereby providing an environmentally compatible, safe and efficacious control of pests and pathogens.

The complete disclosure of all patents, patent documents, and publications cited herein are incorporated by reference. The detailed descriptions and examples herein have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and

described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

Example 1. Inhibition of Growth of Plant Bacterial Pathogens, *Erwinia sp.* and *Xanthomonas sp.* bacteria, using the Terpene β-ionone.

Inhibition of growth of *Erwinia sp.* and *Xanthomonas sp.* bacteria using the terpene β-ionone was tested. A dose response curve for inhibition of growth of plant bacterial pathogens by the terpene, beta-ionone was obtained. The inhibitory response caused by the β-ionone was then quantified measuring a zone of inhibition around a treated filter disk.

The following sources of bacteria were used for the tests:

Table 1. Description of Bacteria used for the Experiments

Bacteria	Identifier	Source of Culture
Xanthomonas vesicatoria races	*"races"*	*AgriPhi*
Xanthomonas campestris pv juglandins	*sjv624*	*Isolated from infected walnuts, Patterson, CA*
Xanthomonas campestris pv juglandins	*X-30*	*Jim Adaskaveg, Univ. of Calif. Riverside*
Erwinia amylovora	*sjv626*	*Isolated from Gala apple trees, Patterson, CA*
Xanthomonas campestris	*mad834*	*Isolated from carrots, Madras, OR*

Each of the strains of bacterial tested were grown overnight in a liquid medium (Luria broth) to stationary phase according to change in optical density measured at 550 nm.

Bacterial cells were isolated by centrifugation and diluted in 25 mM phosphate buffer (pH 6.8) to a concentration of 1 x 10^9 cells/ml, then 0.1 ml of cells were pipeted onto petri dishes containing a selective media that supported growth of both *Erwinia* and *Xanthomonas* species. The bacterial suspension was spread uniformly across the plate and allowed to dry for 30 minutes. The test substance was supplied by serially diluting a solution of 85% β-ionone in 15% reagent grade ethanol with the required volume of reagent grade ethanol to create test solutions of 0, 33, 100, and 333 ppm, then dipping a 1/4 inch sterile filter paper disk into the test solution and placing it in the middle of the petri plate. Cultures were grown for 24 hours at 28° C, and rated both for the size of the zone of inhibition and the degree of growth inhibition in the zone.

β-ionone, dissolved in ethanol and applied to the surface of the petri dish inhibited growth of *Xanthomonas sp.*, and *Erwinia amylovora* at concentrations between 33 and 333 ppm. Application of the filter disk physically cleared the area around the disk of bacteria. However, the distance between the edge of the filter disk and confluent growth of bacteria, and the inhibition of bacterial growth in the zone of inhibition increased with β-ionone concentrations. This suggested that the response was not the result of either the application of the alcohol solvent or the physical washing of bacteria from this area of the plate as the disk was applied.

Table 2. Inhibition of Bacterial Growth by β-ionone.

Bacterial Strain	*Measurement*	*0*	*33*	*100*	*333*
Xanthomonas vesicatoria races 1, 2	*Zone (mm)*	*1*	*3*	*4*	*4*
	Growth (1-5)	*4*	*1*	*1*	*2*

Xanthomonas campestris pv juglandis	*Zone (mm)*	*0*	*N.D.*	*5*	*N.D.*
	Growth (1-5)	*5*	*N.D.*	*1*	*N.D.*
Erwinia anylovora	*Zone (mm)*	*1*	*1*	*2*	*3*
	Growth (1-5)	*3*	*2*	*2*	*1*

Zone = average distance between edge of filter disk and confluent bacterial growth

Growth = estimate of growth in zone of inhibition 1 - none and 5 = confluent growth

N.D. = no data

Dosage is given in parts per million

Example 2. Determining the Specific Insecticidal Properties of β-ionone.

(a) A commercial formulation of β-ionone and limonene consisting of 5% β-ionone -80 (80% β-ionone and 20% α-ionone) and 95% d-limonene was used to determine the insecticidal properties of β-ionone. The following insects were sprayed with the formulation and were instantly killed:

Wasps (yellow jackets)
Cockroaches
Palmetto Bugs
Spiders

The following insects were sprayed with the same formulation, but wiggled for a few seconds (less than 1 minute) before dying:

Ants
Millipedes
Fire Ants were sprayed with the formulation and died within 60 seconds

(c) The following insects and arthropods were sprayed with a formulation containing 10% β-ionone-80 (80% β-ionone and 20% α-ionone) and 90% limonene. The results are as described:

Fire ants, hornets, wasps, flies, mosquitoes, palmetto bugs, spiders, cocroaches, army ants: Died instantly.

Grasshopper: No immediate or short term effect

(d) Fire ants were sprayed individually with β-ionone and limonene. β-ionone and limonene did not have any effects on fire ants when used individually.

Example 3. The use of a β-ionone-limonene formulation as a Fly Repellant

A β-ionone-limonene formulation consisting of 40% β-ionone-80 (80% β-ionone and 20% α-ionone), 45% d-limonene, 10% Tween-80, and 5% Isopropanol was diluted 1:1 in water and sprayed on two animals, a horse and a pony that had a preponderance of horse flies on their body surface. The horses were observed for a duration of 1 hour. Upon the administration of the β-ionone formulation, the flies flew away from the horse and the pony. At the end of 1 hour, no flies were observed on either animal.

Example 4. The use of a β-ionone-limonene formulation on lizards, chameleons and frogs

A β-ionone-limonene formulation consisting of 10% β-ionone-80 (80% β-ionone and 20% α-ionone) and 90% limonene was tested on black lizards, chameleons, tree frogs, and toads. Each animal was sprayed until saturated and dripping with the formula. There were no immediate or short term negative effects on the animals.

Example 5. The use of β-ionone as a Snake Repellant

A β-ionone-limonene formulation consisting of 10% β-ionone-80 (80% β-ionone and 20% α-ionone) and 90% limonene was tested on yard snakes. Upon the administration of the β-ionone-limonene formulation, the snakes moved away rapidly. Administration of the formulation appeared to cause some pain and burning to the snakes. The snakes were repelled and did not return to the place of administration of the β-ionone-limonene formulation.

Example 6. Determining the Specific Herbicidal Properties of β-ionone.

All the following studies were conducted with a β-ionone formulation consisting of 5% β-ionone-80 (80% β-ionone and 20% α-ionone) and 95% d-limonene, unless stated otherwise.

Study I: The Weed Killing Properties of β-ionone

(i) Various weeds commonly found in a suburban yard in Tampa, FL were used to test the weed-killing properties of β-ionone. Two varieties of round leaf creeping ground cover plant (Plants A & C), a single broadleaf weed (B), a 7" tall, thin , grassy plant that eventually sprouts burr-type protrusions (D), were selected and sprayed with the β-ionone formulation. Photographs of each plant type were taken pre-spray, and at 1 hour, 24 hours and 72 hours post spray. The spraying occurred mid-day during the month of June. The spray was applied so that the entire surface of the plant was covered.

Within 1 hour of spraying, all plants turned a darker green and wilted. At 24 hours, all were brown and wilted. At 72 hours, all were brown, wilted and had dried. Plant D was lost in the grass after spraying. However, at 72 hours the dead spot in the grass was evidence of where it had been. See Figures for Study 1.

(ii) The β-ionone formulation was sprayed on common weeds found in flower beds. These included dandelions, crab grass, and St. Augustine grass. The leaves and stalks in the dandelions, and crab grass turned dark green within 20 minutes of spraying. The plants died within 24 hours of spraying. The St. Augustine grass died within 12 hours of spraying.

This formulation was then tested on a flower bed. The flower bed was sprayed with the formulation using a standard garden sprayer. The weeds were killed but the adjacent plants were not affected. It was determined that this formulation would kill the adjacent plants only through direct administration of the formulation on the plants. Thus, this formulation may also be used as an edger. See Figure for "Miscellaneous trials - Edging."

(iii) The β-ionone formulation was sprayed on the soil in flower beds to kill both ants and weeds. 48 hours after spraying, two hibiscus (1 gallon size) plants were planted in the soil. No negative side effects were observed from being planted in the same soil.

(iv) Poison Ivy was sprayed thrice with a 1.5 ml spray of the β-ionone formulation. The poison ivy died within 24 hours. Upon subsequent observation, there was no evidence of regrowth up to 120 days post spraying.

(v) A 3 foot mesquite tree was sprayed 4 times with a 1.5 ml spray of the β-ionone - formulation. The plant died within 24 hours. It is theorized that the formulation accelerates the plant's maturation. Observations indicate that the plant becomes greener and greener until it turns black. After this stage, the leaves fade to yellow or white and the plant dies.

Study II: Effects of Refrigeration

A sample of sod consisting of one plug of Floratam (St. Augustine grass) in good condition, was sprayed with the a β-ionone formulation placed in a household refrigerator (~40°F) and checked at 1, 4, 6, and 24 hours post spray. The formula had no effect on the viability of the sod.

The same plug of sod was then placed outside in typical Tampa, FL June weather (85-95°F). The blades of grass turned brown and appeared dead within 24 hours. The sod was not re-sprayed with the formula. Four days after the grass appeared dead, the plug of sod was watered and watched for another week. No sign of plant life reappeared. This indicated that the roots were killed as well. See Figures for Study II.

Study III: Light and Temperature

Four plugs of Floratam sod as described in Study II that had partially dried out were used in this study. Each plug was sprayed with the β-ionone formula and subsequently placed in each of the following conditions and monitored at 1, 3, 7.5 and 24 hours post spraying.

Sunlight, above 85°F

Darkness, above 85°F

Sunlight, 75°F

Darkness, 75°F

No changes took place in the sod over a 48 hour period. See Figures for Study III for results at 7.5 and 24 hours post spraying.

The activity of the formulation described above was tested by applying a sample from the same bottle on a broadleaf weed in a suburban Tampa, FL yard. The weed

turned dark green and began wilting within 10 minutes after spraying. Thus, it was concluded that the formula used on the sod plugs in this study was active, but that the application of water prior to the spraying with the formula acted as a protective barrier for the plant against the effects of the terpene formula.

Study IV — Effects of Light and Temperature without prior Application of Water

Study III was repeated with new Floratam plugs that were not watered prior to the application of the β-ionone formulation. The plug exposed to sunlight at greater than 85°F showed signs of wilting at 24 hours. The formulation used in study III did not seem to have much effect on the other plugs. However, it may be concluded that sunlight and higher temperatures speed up the rate at which the formulation works.

The light and temperature trials were further conducted on a variety of small, round leaf ground cover weeds. These were sprayed with the terpene formulation and left for 24 hours. During this incubation period the high temperature for the day was around 60°F. At 24 hours the weeds were dark and wilted. The weeds died and turned brown within 72 hours. While the cooler temperature of 60°F may have lowered the activity of the terpene formulation, it still shows activity at 60°F.

In another study, small clover type weeds contained in a flowerpot were sprayed with the formula. They appeared dead within 24 hours. The plant was then watered and observed on a regular basis. No signs of life reappeared during the next 3 weeks after which it was discarded. See Figures for Study IV.

Study V: The Effect of β-ionone terpene formula on North Georgia Weeds

Woodland weeds, native to Northeast Georgia, and Kudzu were sprayed with a β-ionone formula consisting of 5% β-ionone-80 (80% β-ionone and 20% α-ionone) and

95% d-limonene and checked at 24 and 72 hours. The weeds sprayed were small maple and oak saplings, grass and poison ivy, brambles, a fuzzy leafed plant and thistle, and Kudzu.

At 24 hours post spraying, the Kudzu, which had already wilted at 6 hours, was very wilted and brown, the woody stemmed plant and brambles exhibited signs of wilting and brown edges on the leaves and the grasses and poison ivy were turning brown. The fuzzy leaf plant and thistle showed no effects of being sprayed.

At 72 hours post spraying, the Kudzu was brown and dead, the saplings had decidedly brown and damaged leaves, but the plant as a whole seemed to be surviving, the brambles were very brown and wilted and the grasses and poison ivy were dead. The fuzzy leaf weed and thistle were just beginning to show some signs of yellowing on the edges of the leaves where they had been sprayed with the formula. See Figures for Study V.

It can be concluded that woody stemmed plants, especially those with waxy leaves, are less susceptible to the killing effects of the β-ionone formulation. It also appears that coverage may be a factor. Almost all of the leaves on the plant should be sprayed in order to kill the entire plant. Fuzz on leaves (including the thistle) seems to have some protective properties for the plant against the formula. However, eventually the formula has a negative effect on the plant. Kudzu is very susceptible to the effects of the terpene formula.

Example 7. Determining the Effects of β-ionone on the Growth Rate (Fluorescence) of Flowering Plants

Experiments were conducted to determine if β-ionone can slow the growth rate (Fluorescence) of flowering plants. Identical azalea plants, approximately 10 days from blooming, were divided into three groups of two plants each. They were each sprayed once with 50 grams of the following formulations:

1. Water (control);
2. 0.25% β-ionone mixture in water; and
3. 0.5% beta-ionone mixture in water.

The β-ionone mixture was 85% β-ionone (98% purity) and 15% Tween-80 to make it miscible in water.

It was observed that the plants sprayed with β-ionone bloomed approximately one week later than the control plants (plants sprayed with water) and held their blooms for the same period as the control. The 0.5% β-ionone mixture in water delayed blooming one day more than the 0.25% β-ionone mixture in water The experiment was repeated twice, and yielded similar results.

Example 8. The Use of β-ionone as a Plant Growth Regulator

A formulation consisting of 40% β-ionone, 45% d-limonene, 10% Tween-80, and 5% isopropanol was diluted 1:100 in tap water, and sprayed on a lawn consisting of St. Augustine's Grass. It was observed that the grass turned greener, and that the

formulation had a growth regulating effect on grass. This formulation was also tested on Bahiia grass commonly found in Florida. A similar effect was observed.

The diluted formulation was also sprayed on adjacent plants like Hibiscus, Chinese palms, Boxwoods, Azaleas, etc. This formulation displayed no toxicity towards these plants.

Formulations may be diluted at dilution factors ranging from 20 to 200 with similar activity. However, when the formulation is diluted at a dilution factor of less than 20, the diluted formulation becomes thick and turgid and is difficult to use.

Example 9. Management of Walnut Blight

Studies on the epidemiology and management of walnut blight were conducted by the Department of Plant Pathology, University of California, Riverside. In these epidemiological studies, temperature, leaf wetness, relative humidity, and walnut blight incidence were monitored using field dataloggers for orchards in Butte County and Tehama County, CA. The treatments evaluated and compared included a formulation (694-B) consisting of 60% β-ionone, 15% Tween-80 and 25% α-terpenol, activated host resistance compounds (*e.g.*, Actigard), and zinc-based (Zinc 7-$Ca(OH)_2$, Zinc 7.5-$Ca(OH)_2$) compounds. These were compared to copper-based (Kocide 101) compounds and check treatments. In the case of management of walnut blight in California, the extensive use of copper-based treatments in California for more than 25 years is attributed to the development of resistant populations of the walnut blight organism. Copper-maneb or copper-mancozeb mixtures were more effective than copper alone for managing bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria*.

Actigard is a product that is not bactericidal but functions by stimulating natural host defenses in the plant.

The following chemicals were evaluated in hand spray trials on walnut fruit in the field: a natural bactericidal product (B-694), an activated host resistance compound (*e.g.*, Actigard), polyhexamethylene biguanide (PHMB), and zinc-based compounds (Zinc 7-$Ca(OH)_2$, Zinc 7.5-$Ca(OH)_2$). All treatments were applied to run-off at a rate of 400 gal/A. Walnut fruits were treated, air-dried, inoculated with aqueous suspensions of *X.c.*pv. *juglandis* ($1x10^6$ cfu/ml), bagged for 24 hrs, and evaluated after indicated time intervals after inoculation.

It was determined that all treatments were effective and significantly reduced the incidence and severity of walnut blight as compared to non-treated trees. 694-B was also efficacious in reducing incidence and severity of disease as shown in the table below.

Table 9.1

			Product	Walnut Blight Incidence (%)					
No.	Trade	Common	Rate	Evaluation after Treatment				Severity	
	Name	Name	(ppm)	4 wk	LSD	5 wk	LSD	Les./Fruit	LSD
1	Untreated			65.8	a	88.9	a	0.5	a
2	694-B	--------	100	36.9	b	53.9	b	4.0	b
3	Actigard		75	29.1	b	49.2	b	3.3	b
4	Zinc-7 /Ca(OH)2	Zinc Lignosulfate	2%-500	45.3	ab	65.0	ab	5.2	b
5	Zinc-7.5 /Ca(OH)2	Zinc Citrate	2%-500	29.0	b	58.6	ab	4.5	b

LSD = Least significant difference

Thus, treatment with 694-B is efficacious in reducing the incidence and severity of disease, and is comparable or superior to the other treatments. See Table 9.1. A significant decrease in walnut blight incidence is observed in treated trees in comparison to the untreated trees.

Example 10. Treatment of Brown Rot in Fruit Trees using 694-B

Studies were conducted to determine the efficacy of new or existing fungicides for the control of brown rot, blossom blight and fruit rot. The fungicides evaluated on Casselman plums and Fairtime peaches were Maxim (fludioxinil), Break (a DMI compound), Topsin-M (thiophanate-methyl), and 694-B (1000 ug/ml). All treatments were used in combination with 25% wax/oil emulsions (Decco 251). Fungicides were applied using a T-Jet application system and rates were based on amount of fungicide per 200,000 lb. of fruit. Four replications of 12 fruit/rep were used for each treatment. Lesion diameter and disease incidence (number of infected fruit/total fruit) were recorded daily. Treatments consisted of 3-4 replications with 8-10 fruit/rep. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS.

Results showed that using inoculated and treated fruit, Maxim and Break significantly decreased the incidence and severity of each post-harvest decay (See Example 10 - Figures 1A, 1B). Other materials such as Ca-based clay and 694-B were ineffective against all decays. Topsin-M was effective against gray mold but not Rhizopus rot or brown rot. Using treated and inoculated fruit, most materials were less effective (See Example 10-Figures 2A, 2B). Maxim, Break, Ca-based clay, and B-694 significantly reduced brown rot. Topsin-M was only effective for gray mold.

On Casselman plum and Fairtime peach, additional packing line tests with fungicides were conducted (See Example 10-Figures 3A, 3B, 4A, 4B). On plums, when treatments were applied to wound-inoculated fruit, most treatments were effective for management of brown rot including the experimental compound 694 B and the other fungicides including Break, Elite, Elevate, Rovral, Topsin-M, and Maxim (See Example 10-Figures 3A, 3B). Regardless of inoculation procedure, all of the fungicides were effective for brown rot management.

Example 11. Evaluation of 694 B-3 Formulation for Control of Gray Mold of Pear

D'Anjou pear fruit were harvested at commercial maturity and stored at -1°C until further use. Fruit were surface-sterilized in 100 ppm a.i. sodium hypochlorite and rinsed with water. Each fruit was wounded at two locations with a metal tool that made a circular hole 6 mm diameter and 3 mm deep, similar in size and shape to natural stem puncture wounds.

Conidia of *Botrylis cinerea* isolate 62 were harvested from 15-day-old cultures grown on acidified potato dextrose agar. The experimental fungicide 694 B-3 was prepared in water at concentrations of 100, 250, 500, and 1000 ppm a.i. Spores of *B. cinerea* were added to the fungicide suspensions to obtain a final concentration of 4,000 spores per ml. Wounded pear fruit were dipped in the fungicide-spore suspensions (15 fruit per fungicide concentration) for 30 seconds, then placed on cardboard trays. Trays were placed in boxes lined with perforated polyethylene fruit bags and incubated at 22°C. Disease incidence and severity were evaluated after 1 week. Results are as follows:

Table 11.1

	Results	
694 B-3 (μg/ml)	% wounds infected	Avg. lesion diameter (mm)
0	97	53
100	100	55
250	100	54
500	100	52
1000	100	49

Example 12. Evaluation of 694-B Formulation for Control of Spore Formulation

The following experiments were conducted:

Experiment 1. Spores washed from plates of *Penicillium expansum*, *Cladosporium herbarum*, *Alternaria spp.*, and *Phialophora malorum* were placed in water containing 0, 100, 300, and 500 ppm of the 694-B formulation. The 694-B formulation consists of 60% β-ionone, 15% Tween-80, and 25% α-terpenol. Drops of solution were examined under the microscope for spore germination after 1, 3, and 5 days. No germination was observed at any treatment level, including 0 ppm, apparently due to lack of nutrition to stimulate germination. After 3 days, drops of solution from each treatment were placed on acidified potato dextrose agar (APDA), a standard growth medium for the fungi. No growth was seen with *Cladosporium herbatum* at concentrations of 100 ppm or greater. The fungus grew normally from 0 ppm controls. All other fungi grew from all

treatments, although colony development by *Penicillium expansum* was slower with increasing treatment concentration. No pH adjustment performed in this experiment.

Experiment 2. Poison food test. Potato dextrose agar was prepared in the autoclave, and the 694-B formulation as described in Example 12- experiment 1 was added to molten agar to give final concentrations of 0, 100, 300, and 500 ppm. Agar was poured into petri dishes, and test fungi were plated on the agar surfaces after hardening at a dosage of approximately 50 spores per plate. After 4 days, the number of colonies which developed on each plate were counted. No differences were observed among treatments.

Experiment 3. The above experiment was repeated, except the agar was allowed to cool prior to addition of the 694-B formulation as described above. Lawns of fungus colonies developed on the controls, too numerous and overlapping to count. An estimate was made of the differences in the amount of the surface of the petri dish covered with fungus. No difference with *Phialophora* or *Alternaria* was observed. Percentages of colony development for the other fungi were:

	ppm			
	0	**100**	**300**	**500**
Cladosporium	100	60	30	5
Penicillium	100	90	85	70

Experiment 4. Solutions were prepared of the test concentrations used in experiment 3, and the pH was adjusted from around 6.5 to 7.8 with baking soda. Spores of each pathogen were introduced (*botrytis cinerea* was substituted for *Alternaria*). After 1 hour in solution with periodic agitation, samples were drawn from each solution and diluted by a factor of 10,000 in water. Samples of the diluted solution were then plated on APDA. After 4 days, the number of colonies developing were counted. The average numbers of colonies per 3 replicate platings are shown below.

	ppm			
	0	100	300	500
Penicillium	11	19	15	18
Cladosporium	2.3	1.7	0.7	1.3
Botrytis	0.3	2.0	0.7	1.7
Phialophora	81	37	49	38

Example 13. Efficacy of Using a Terpene Mixture to Kill Bacterial Contaminants on Garden Vegetables and Fruits

Tests were conducted to investigate the efficacy of using a terpene mixture to kill bacterial contaminants on garden vegetables and fruits. These fruits and vegetables are often contaminated by *E.Coli*, Salmonella, and Shigella bacteria when handled by field workers. The tests were specifically conducted to ascertain the effect of the products on reducing or eliminating contamination.

A mixture of a material FT6 or tall oil was used in conjunction with a solution of 85% β-ionone and 15% terpeniol. This combination had little or no effect on controlling the contamination.

However, when a mixture of 85% β-ionone and 15% terpeniol was combined with polysorbate 80 or a silicon surfactant such as Silvet, success was achieved. The materials could be used as sprays, washes or as fumigants with equal efficacy. 2.5 to 50 ppm was lethal to the contaminating bacteria, reducing their recovery from the fruit or vegetables by 4 to 5 logs of bacterial pathogens. Contact time between the mixture and the market fruit and/or vegetables was critical, requiring 10 to 12 minutes for efficacy.

Example 13. Effect of B-ionone and B-ionone/α-terpineol on the Survival of *Escherichia Coli* in Solution

In treatement 1, 100 ppm of β-ionone in 0.15% (v/v) Tween 80 was added to a suspension of stationary phase *Escheria coli* containing an average of $2.9x10^3$ cells per millimeter of solution. At intervals after mixing, aliquots were removed and plated on Luria Broth plates containing 1.5% agar, and incubated for 24 hours at 37°C prior to counting.

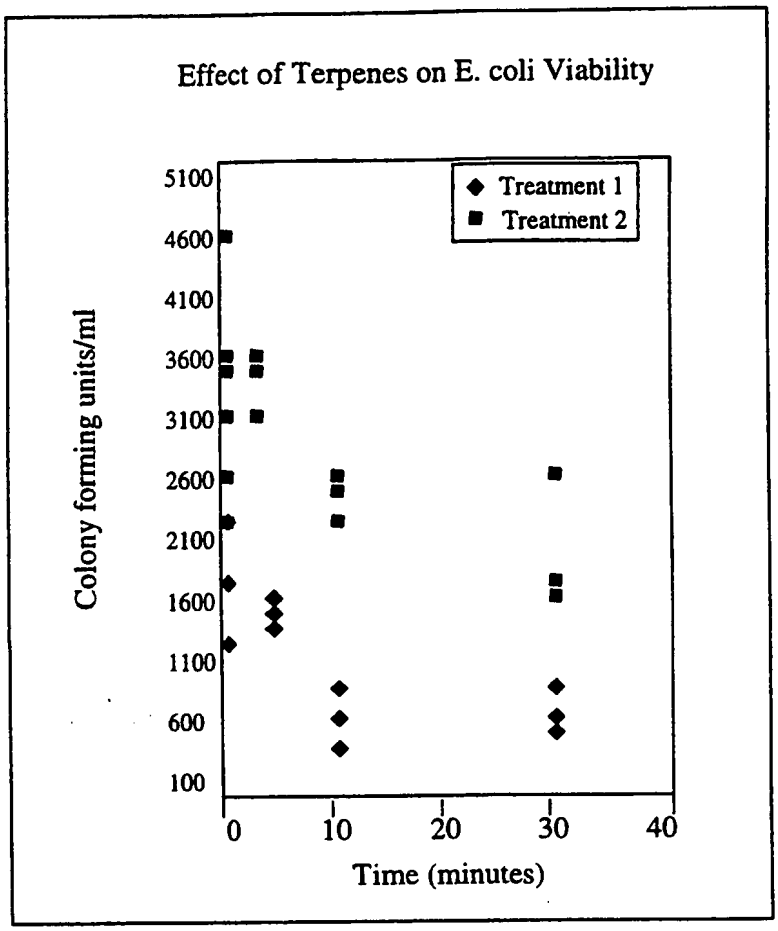
In treatment 2, 100 ppm of β-ionone plus 25 ppm α-terpineol encapsulated in a lecithin liposome preparation were added to stationary phase *Escheria coli* containing an average of $2.9x10^3$ cells per millimeter of solution. At intervals after mixing, aliquots were removed and plated on Luria Broth plates containing 1.5% agar, and incubated for 24 hours at 37°C prior to counting.

A positive control treated with 1.% Tween 80 (time 0) showed no significant change in counts over a thirty minute period.

The data for these experiments is set forth and plotted below:

Effect of B-ionone and B-ionone/α-terpineol on *Escherichia Coli*

Time (min.)	Treatment 1 (colony forming units/ml)	Treatement 2 (colony forming units/ml)
0	2.20E+03	2.20E+03
0	2.70E+03	2.70E+03
0	3.70E+03	3.70E+03
0.5	1.80E+03	3.50E+03
0.5	2.20E+03	3.10E+03
0.5	1.30E+03	4.50E+03
3	1.50E+03	3.50E+03
3	1.60E+03	3.10E+03
3	1.40E+03	3.70E+03
10	6.03E+03	2.40E+03
10	3.70E+03	2.10E+03
10	9.10E+03	2.60E+03
30	5.40E+03	1.70E+03
30	8.30E+03	2.60E+03
30	6.20E+03	1.80E+03


Effect of Terpenes on E. coli Viability
Treatment 1
Treatment 2
Colony forming units/ml
5100
4600
4100
3600
3100
2600
2100
1600
1100
600
100
0
10
20
30
40
Time (minutes)

WHAT IS CLAIMED IS:

1. A composition comprising:

(a) about 1-99.9 %, by volume, a reagent comprising an ionone composition, another terpene, a surfactant, and an alcohol; and

(b) water.

2. The composition of claim 1, wherein the reagent comprises by volume:

(a) about 1-90% an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone;

(b) about 1-90% another terpene, selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene;

(c) about 1-30% a surfactant, selected from the group consisting of polysorbate, silicons,and tween based surfactants; and

(d) about 1-10% alcohol selected from the group consisting of isopropyl and ethyl alcohol

3. The composition of claim 1, wherein the reagent comprises by volume:

(a) about 10-40 % an ionone composition containing β-ionone and α-ionone

(b) about 10-50 % DL-limonene;

(c) about 2-20 % tween 80; and

(d) about 1-10 % isopropyl alcohol.

4. The composition of claim 3, wherein the ionone composition comprises by volume:

(a) about 60-80% % β-ionone; and

(b) about 20-40% α-ionone.

5. A method to repel flies which comprises administering a composition comprising:

(a) about 1-99.9 %, by volume, a reagent comprising an ionone composition, another terpene, a surfactant, and an alcohol; and

(b) water.

6. The method of claim 5, wherein the reagent comprises by volume:

(a) about 1-90% an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone;

(b) about 1-90% another terpene, selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene;

(c) about 1-30% a surfactant, selected from the group consisting of polysorbate, silicons,and tween based surfactants; and

(d) about 1-10% alcohol selected from the group consisting of isopropyl and ethyl alcohol

7. The method of claim 5, wherein the reagent comprises by volume:

(a) about 10-40 % an ionone composition containing β-ionone and α-ionone

(b) about 10-50 % DL-limonene;

(c) about 2-20 % tween 80; and

(d) about 1-10 % isopropyl alcohol.

8. The method of claim 5, wherein the ionone composition comprises by volume:

(a) about 60-80% % β-ionone; and

(b) about 20-40% α-ionone.

9. A method to regulate plant growth which comprises administering a composition comprising:

(a) about 1-99.9 %, by volume, a reagent comprising an ionone composition, another terpene, a surfactant, and an alcohol; and

(b) water.

10. The method of claim 9, wherein the reagent comprises by volume:

(a) about 1-90% an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone;

(b) about 1-90% another terpene, selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene;

(c) about 1-30% a surfactant, selected from the group consisting of polysorbate, silicons,and tween based surfactants; and

(d) about 1-10% alcohol selected from the group consisting of isopropyl and ethyl alcohol

11. The method of claim 9, wherein the reagent comprises by volume:

(a) about 10-40 % an ionone composition containing β-ionone and α-ionone

(b) about 10-50 % DL-limonene;

(c) about 2-20 % tween 80; and

(d) about 1-10 % isopropyl alcohol.

12. The method of claim 9, wherein the ionone composition comprises by volume:

(a) about 60-80% % β-ionone; and

(b) about 20-40% α-ionone.

13. An insecticide composition comprising

(a) 1-20%, by volume, an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) 80-99% another terpene selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene.

14. The insecticide composition of claim 13, wherein the ionone composition comprises by volume:

(a) about 60-80% % β-ionone; and

(b) about 20-40% α-ionone.

15. A method to repel insects, arthropods, and snakes which comprises administering the insecticide composition of claims 13 or 14.

16. A method to render inactive insects and arthropods which comprises administering the insecticide composition of claims 13 or 14.

17. A method to kill insects and arthropods which comprises administering the insecticide composition of claims 13 or 14.

18. A herbicide composition comprising

(a) 1-20%, by volume, an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) 80-99% another terpene selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene.

19. The herbicide composition of claim 18, wherein the ionone composition comprises by volume:

(a) about 60-80% % β-ionone; and

(b) about 20-40% α-ionone.

20. A method for killing weeds selected from the group consisting of broad leaf plants, grasses, brambles, mesquite, and poison ivy, said method comprising treating the weeds with the herbicide composition of claim 18.

21. A method for selectively killing weeds found in turf, said method comprising treating the turf area with the herbicide compositions of claims 18.

22. A method for killing weeds selected from the group consisting of broad leaf plants, grasses, brambles, mesquite, and poison ivy, said method comprising treating the weeds with the herbicide compositions of claims 18.

23. A method for killing weeds found in fields by pre-treating the fields prior to crop planting with the herbicide compositions of claims 18.

24. A method for killing bacteria selected from a group consisting of vanthomillus, pseudomonas, xanthomonas, and erwinia, said method comprising treating the bacteria with a reagent comprising:

(a) an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) another terpene, selected from the group consisting of DL-limonene, dipentene, citral, α-terepenol, terpineol, and pinene.

25. The method of claim 24, said method comprising treating the bacteria with a reagent further comprising a surfactant, selected from the group consisting of polysorbate and tween based surfactants.

26. The method of claim 24, said method comprising treating the bacteria with a reagent further comprising an alcohol selected from the group consisting of isopropyl and ethyl alcohol.

27. A method for killing fungi selected from a group consisting of candidia, microspora, brown rot fungus, walnut blight fungus, gray mold, rhizopus rot, and brown rot, said method comprising treating the fungi with a reagent comprising:

(a) an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) another terpene, selected from the group consisting of DL-limonene, dipentene, citral, α-terepenol, terpineol, and pinene.

28. The method of claim 27, said method comprising treating the fungi with a reagent further comprising a surfactant, selected from the group consisting of polysorbate, silicons, and tween based surfactants.

29. The method of claim 27 , wherein the reagent is administered to fields prior to crop planting.

30. A method for selectively killing fungi found in soil for turf area, said method comprising treating the soil with a reagent comprising:

(a) an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) another terpene, selected from the group consisting of DL-limonene, dipentene, citral, α-terepenol, terpineol, and pinene.

31. The method of claim 30, said method comprising treating the soil with a reagent further comprising a surfactant, selected from the group consisting of polysorbate, silicons, and tween based surfactants.

32. A method for slowing the growth rate of flowering plants, said method comprising treating the flowering plants with a reagent comprising:

(a) an ionone composition containing at least one ionone, selected from the group consisting of β-ionone, α-ionone, and pseudo-ionone; and

(b) another terpene, selected from the group consisting of DL-limonene, dipentene, citral, α-terepenol, terpineol, and pinene.

33. The method of claim 32, said method further comprising treating the flowering plants with a reagent further comprising a surfactant selected from the group consisting

of polysorbate, render inactive insects and arthropods silicons, and tween based surfactants.

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(71) Applicant: THE VAN KAMPEN GROUP, INC. [US/US]; 881 East 5550 South, Ogden, UT 84405 (US).

(72) Inventors: VAN KAMPEN, Kent; 1008 Lake Winds Drive, Hoover, AL 35244 (US). EDWARDS, Bobby, G.; 19448 FM 2115, Salado, TX 76571 (US). FRANKLIN, Lanny, U.; 5170 Chemin de Vie, Atlanta, GA 30342 (US). HYMAS, Theo; 13855 Via Encantado, Valley Center, CA 92082 (US).

(74) Agents: LAURENZO, Brian, J. et al.; Dorsey & Whitney LLP, Suite 3900, 801 Grand, Des Moines, IA 50309 (US).

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(54) Title: TERPENE COMPOSITIONS AND METHODS OF USE

(57) Abstract: The use of β-ionone (a naturally occurring compound, chemically classified as a terpene) alone or in combination with other terpenes, biologicaly actives substances, and surfactants to inhibit growth, proliferation, impede such, render inactive or kill certain classes of plants, bacteria, fungi, yeast, viruses, parasites and insects. Wherein varying combinations of β-ionones in the above mentioned combinations have been demonstrated to kill braod leaf plants and grasses, impede fluorescence, budding, rooting of certain plants; whereas such combinations kill, impede metamorphosis, stun, incapacitate selected insects and parasites; wherein β-ionone kills anaerobic bacteria that are pathogens of live stock and man; the combinations kill vanthomillus, pseudomonas and other pathogenic bacteria affecting plants; wherei n the combination kills fungi, specific candidia, microspora and others infecting man and animals, the invention serves multiple functions as a means to control or kill certain pests or pathogens. β-ionone is non-toxic to humans and when exposed to the natural environment undergoes breakdown (Degradation) into carbon dioxide and water providing an environmentally compatible, safe and efficacious control of pests and pathogens.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 504/348; 514/690

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,832,464 A (HENNART) 27 August 1974 (27.08.1974), see columns 3 and 9.	1-33
Y	US 5,693,344 A (KNIGHT et al) 02 December 1997 (02.12.1997), see columns 2-4 and claims.	1-33
Y	US 5,696,171 A (RUPP et al) 09 December 1997 (09.12.1997), see columns 2-5.	1-4, 13, 14, 18, 19
Y	GB 1 295 567 A (PFIZER, INC.) 08 November 1972 (08.11.1972), see entire document.	1-33
Y	Derwent Database, Accession No.: 1995-196675, ST KAGAKU KK, JP 07-112907 A, 02 May 1995, see entire abstract.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search 15 JUNE 2000	Date of mailing of the international search report 30 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer S. Mark Clardy Telephone No. (703) 308-1235

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/04808

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST: (pseudo)ionone, terpene, limonene, dipentene, citral, terpineol, pinene; polysorbate, silicones, tween; flies, insects; herbicid-, insecticid-, microbicid-, antibacterial, antimicrobial-, fungicid-